Congener Specific Determination of Polychlorinated Naphthalenes in Sediment and Biota by Gas Chromatography High Resolution Mass Spectrometry

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Abstract

An isotope dilution congener-specific method for the determination of the most abundant and most toxic polychlorinated naphthalenes (PCNs) was developed using gas chromatography with high resolution mass spectrometry (GC-HRMS). The method was used to determine the concentration of 24 target congeners and total PCN concentrations in fish and sediment samples. Tissue samples were extracted using pressurized liquid extraction (PLE) and sediment samples were extracted using Soxhlet extraction. Sample extracts were cleaned up using either a manual two-stage open column procedure or an automated FMS Power Prep System with multi-analyte and multi-sample capability using a three-column cleanup procedure. Sediment extracts were cleaned up with a dual open column cleanup technique involving the use of both a multi-layered silica (silver nitrate/acid/base/neutral silica) column followed by column containing carbon-activated silica. Fish tissue extracts were cleaned up on the automated system involving the use of a high capacity ABN (acid/base/neutral column), carbon celite column, and a basic alumina column. The method is capable of producing instrument detection limits (IDLs) between 0.06 and 0.13 pg for each PCN (on column), with method detection limits (MDLs) for the fish extracts ranging from 1.3 to 3.4 pg/g (wet weight) and 0.46 to 1.2 pg/g (dry weight) for sediments. The average accuracy of 34 spiked fish samples analysed over a period of several months was 100% with a precision (%RSD) of 12%. Similarly, the average accuracy for 28 spiked sediment samples was 104% with a precision (%RSD) of 12%.The application of the method to environmental samples was demonstrated through the analysis of sediment and fish samples obtained from Lake Ontario, Canada. The method is used both for the determination of 24 PCNs and to perform non-targeted screening for the remaining 51 PCN congeners, which are included in the total PCN quantification result. It is currently one of the most comprehensive and accurate congener-specific methods available and was developed from the existing techniques used for the determination of polychlorinated dioxins and furans to produce high quality data with only minor modifications in the clean-up procedure. It can therefore be readily adopted by other laboratories performing dioxin and POP analyses.

Keywords

Polychlorinated Naphthalenes; PCN; Sediment; Biota; Gas Chromatography; High Resolution Mass Spectrometry

Introduction

Approximately 100,000 different industrial chemicals and chemicals of commerce are currently used today (Muir and Howard, 2006). Many of these compounds are transported to the environment through fugitive emissions and spills or are purposely released into the environment, e.g. through

application of pesticides. Over the past century, humans have become dependent on many chemicals for applications including: increased crop yields, suppression of fires, reduced staining of fabrics, minimizing friction and repulsion of water or grease. A number of these compounds including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were mass produced during the early part of the 20th century. A lack of toxicological assessment and analytical testing capabilities resulted in significant human exposure and environmental damage before their use began to be phased out in the 1970s (O'Sullivan and Megson, 2013). Polychlorinated naphthalenes (PCNs) are one of the first group of industrially synthesized chemicals, however they have received much less attention than PCBs or OCPs. They were first synthesized in 1833 (Laurent, 1833) by reacting molten naphthalene with chlorine gas. A larger scale process to manufacture PCNs as flame retardants was patented in 1909 (Aylsworth, 1909)

Many different types of flame retardants are used today. Halogenated flame retardants are preferred for most applications due to their ability to induce charring, and the chlorine or bromine radicals formed can combine with pre-burn radicals to further suppress ignition. PCNs were one of the first halogenated flame retardants (US Patent 2,028,715) and were used extensively in paper inlays and fabrics (airplane cloth and uniforms) during World War I (Helm et al. 2006). They were also used as cloth based electrical cable insulation, cutting oils, capacitor dielectrics, engine oil additives, electroplating stop-off compounds, in die casting and ship insulation and as wood, fabric and paper preservatives. PCNs were used most extensively during the 1930s to 50s (Jakobsson and Asplund, 2000). Due to reports of a variety of serious health effects including: chloroacne, anorexia, nausea, headaches, weight loss, abdominal pains, insomnia, alopecia, impotence and mortality, their use gradually declined. They were replaced by the supposedly less toxic PCBs.

There are 75 possible PCN congeners which consists of 2 mono-CNs, 10 di-CNs, 14 tri-CNs, 22 tetra-CNs, 14 penta-CNs, 10 hexa-CNs, 2 hepta-CNs and 1 octa-CN. Numbering of the congeners is generally performed according to the system proposed by Wiedmann and Ballschmiter (1993) in which the PCNs are listed from CN-1 to CN-75. Due to their structural similarity to dioxins, several of the 75 congeners bind with the aryl hydrocarbon receptor (AhR) and exhibit dioxin-like toxicity. Congeners such as CN-66 and CN-67 have therefore been recommended for inclusion in the WHO TEF scheme (Hooth et al. 2012, van den Berg et al. 2006). Relative potencies of PCNs compared to 2,3,7,8-TCDD have been suggested for 20 congeners (Puzyn et al. 2007), enabling relative dioxin TEQ values to be calculated.

There is limited information available on the production of PCNs. PCNs were produced as a number of different technical mixtures called Halowax (USA), Nibren wax (Germany), Seekay wax (Great Britain) and Clonacire wax (France). The use of PCNs in North America had been virtually phased out prior to the 1960s before analytical methods sensitive and selective enough to detect human exposure and environmental contamination were developed. For this reason, very few methods for PCN determination exist.

Although most manufacturers of PCNs have stopped their production, many sources still contribute to the environmental PCN load (Liu et al. 2014). These include the release of PCNs from landfills (Jarnberg et al. 1997), combustion related emissions from municipal solid waste incineration (Falandysz, 1998) and chloroalkali processes (Jarnberg et al. 1993). The production of magnesium (Baumann, 1978), copper (Theisen et al. 1993) and aluminium (Vogelgesang, 1986) have also been identified as sources of PCNs to the environment. Very few reports on PCNs and PCBs in the same soils and sediments exist. PCNs are generally present at concentrations one or two orders of magnitude lower than PCBs, however in fish they have been found to contribute to over 10% of the dioxin-like TEQ (Jarnberg et al. 1993; Clement et al, 2012).

PCBs were initially identified as unknown interferences in chromatograms determined by electron capture detection (ECD) for samples analysed for OCPs and due to historical technology limitations PCNs were often misidentified as PCBs (Reiner et al. 2010, Reiner et al. 2013). As a result of the relatively poor chromatographic separation in early chromatograms, and lack of selectivity of the ECD, PCNs were unlikely to be detected in most samples. In areas where PCN levels were elevated, the patterns were often misidentified reported as lower chlorinated PCB technical mixtures such as Aroclor 1221, 1016 or 1232. The use of mass spectrometry coupled to gas chromatography (GC) allowed the specific identification of PCNs and for homolog-specific quantification of PCNs (Erickson et al., 1978). This led to the foundations of many current PCN analytical methods through the determination of PCNs by capillary (high resolution) GC and mass spectrometry (MS) following carbon clean up and fractionation (Jansen et al., 1984). More recently PCNs have been determined by a variety of congener specific methods. Egeback et al. (2004) used GC with high resolution mass spectrometry (HRMS) and performed quantitation using 17 PCN standards and Halowax formulations, with MDLs in the low fg/m^3 range. A multidimensional chromatography method was developed by Hanari et al., (2013) which separated all tetra-, penta- and hexa- CNs in Halowax standards. Li et al., (2014) used triple quadrupole mass spectrometry with GC to quantify 16 PCNs achieving sub pg/m^3 MDLs. Moukas et al. (2016) used LC with atmospheric pressure photoionisation and detection with triple quadrupole mass spectrometry to determine 6 PCNs with MDLs in the low ng/L range. The method presented in this paper builds on these existing methods by using targeted isotope dilution GC-HRMS for the determination of 24 PCNs with low to sub pg/g MDLs in both sediment and biota samples. GC-HRMS enhances sensitivity and selectivity and enables the determination of dioxin-likeTEQ values. The method is also used to perform non-targeted screening for the remaining PCNs, which are included in the total PCN quantification.

Gas chromatography – high resolution mass spectrometry is one of the most sensitive and selective methods used for the determination of persistent halogenated organics and considered the gold standard for the determination of similar compounds to PCNs like dioxins and furans. Few methods have been developed to focus solely on PCNs, however there have been successful examples where PCNs have been determined in combination with other analytes (Liu et al. 2013; Liu et al. 2010; Liu et al. 2009). This paper presents a GC-HRMS based method for the determination of PCNs based on the existing MOECC 3418 method for dioxins and furans with only minor modifications to the sample clean up procedure. The ability to provide a multi-analyte approach has obvious practical applications such as increasing laboratory throughput and reducing costs. This is especially relevant for dioxin analysis where method turnaround times are long and costs are significantly higher than those for other types of analytes (Megson et al. 2016). The method presented is one of the most comprehensive congener-specific methods available and can accurately quantify PCNs that are important for Dioxin-TEQ determinations. It was tested on spiked fish tissue and sediment samples and has been accredited to the ISO 17025 standard.

Experimental

Analytical standards were prepared in-house using certified standards native solutions; PCN-MXA and PCN-MXC and CN-31 (Wellington Laboratories), and individual solutions of CN-42, CN-54, CN-68, CN-70, and CN-74 (Cambridge Isotope Laboratories). The $^{13}C_{10}$ analytical solution was prepared using certified individual standard solutions of $^{13}C_{10}$ labelled CN-27, CN-42, CN-52, CN-67, CN-73, and CN-75 (Cambridge Isotope Laboratories). Method detection limits (MDLs) were determined using nine fortified fish samples and eight fortified sediment samples. Each matrix was analysed as one batch representing within run performance. The MDLs were compared against additional QC samples (fortified matrices) prepared with each sample batch. In order to determine between run performance

data, an additional 34 QC type fortified fish samples and 28 QC sediment samples were compiled over a period of several months. These "between run" samples were analysed for quality control purposes with routine analytical samples on a per batch basis and to determine the reproducibility of the method over time with the instrument in regular use. A native PCN spike solution was added to the blank matrix samples prior to extraction to produce the concentrations listed in Tables 2 and 3 and processed alongside the analytical samples. The method applications and performance are demonstrated through the analysis of a standard solution prepared by the Northern Contaminants Program inter-laboratory study, along with the analysis of sediment and fish samples obtained from different parts of Lake Ontario.

Extraction

The method was developed to quantitatively extract and determine PCNs in fish and sediments. Such extracts may contain many different organic materials including hydrocarbons, humic acids, lipids as well as other interferences and potential analytes like organochlorine pesticides, polyaromatic hydrocarbons (PAH), polybrominated diphenyl ethers (PBDEs), polychlorinated diphenyl ethers (PCDEs), PCBs, PCDDs and PCDFs. Sample extracts were processed to remove many of the compounds that interfere with the analytes of interest; however, additional compounds with similar properties were retained. The method uses isotope dilution for quantification of congeners with a corresponding a $^{13}C_{10}$- labelled PCN (CN-27, CN-42, CN-52, CN-67, CN-73 & CN-75). Internal Standard calibration was used for the remaining congeners without a labelled surrogate.

Sample extraction of tissue was performed using pressurized liquid extraction (PLE – Fluid Management Systems – Waltham MA). A blank fish matrix (Alaskan Pollock, *Theragra chalcogramma*) was thawed and sub-sampled. Three to four grams of tissue (wet weight) were fortified with six $^{13}C_{10}$-PCNs (CN-27, CN-42, CN-52, CN-67, CN-73 & CN-75) (Cambridge Isotope Laboratories, Andover, MA) and the twenty native PCNs specified in Table 2. The sample was mixed with 4 to 5 g of diatomaceous earth (Dionex, Sunnyvale, CA) until it became a free flowing powder which was transferred to a PLE extraction cell. The cell was topped with 1 to 2 g of diatomaceous earth. The samples were extracted with dichloromethane/hexane (10/90) in two stages: the first at 80°C for 10 min and second at 100 °C at a pressure of 1000 psi for 20 min. A soil/sediment matrix (Ottawa sand) was air dried and a sub sample of approximately 5 g was accurately weighed, and fortified with the six $^{13}C_{10}$-PCNs and the native PCNs specified in Table 3. Samples were extracted using Soxhlet extraction performed using 200 mL of toluene refluxed at a rate of approximately 6 cycles per hour for 16 hours (overnight). Prior to sample clean up all extracts were concentrated on a rotary evaporator to approximately 1 mL.

Clean up

The concentrated sediment extracts were subject to clean up using a two-stage open column procedure with the first column containing multi-layered silica (1.5 g 10% silver nitrate / silica (w/w), 1.0 g activated silica, 2.0 g 33% sodium hydroxide/silica (w/w), 1.0 g activated silica, 4.0 g 44% sulphuric acid/silica (w/w), 2.0 g activated silica, and 2.0 g anhydrous sodium sulphate). The column was prepared by rinsing with 50 mL of hexane and discarded, the extract added and eluted with 100 mL of hexane which was collected and subsequently reduced to approximately 1 mL by rotary evaporation. The second column consisted of 0.35 g of 5% Amoco PX21 carbon-activated / silica (w/w), which was prepared by pre-rinsing with 15 mL of acetone, 15 mL of toluene, 30 mL of dichloromethane and 50 mL of hexane all of which was discarded. A round bottom flask was placed under the column and the extract was added and eluted with 40 mL dichloromethane/hexane (25/75) collecting the non-planar Fraction; which includes non-planar compounds like the ortho substituted PCBs, PCDEs and PBDEs. Then the column was inverted to elute the planar fraction with 160 mL

toluene which was collected in a separate flask to isolate the PCNs as well as PCDD/Fs and non-ortho PCBs. Both fractions were reduced to approximately 1 mL using rotary evaporation and then approximately 10 µL of nonane was added to the final extracts before they were reduced to incipient dryness by nitrogen evaporation.

The concentrated fish tissue extract was subject to clean-up on an FMS Automated Power Prep System (PPS) capable of multi-sample and multi-analyte separation (Fluid Management Systems (FMS), Waltham, MA, USA). A three column clean-up procedure involving a high capacity Acid Base Neutral (ABN) Silica Column, Carbon/Celite (0.34 g) Column and a Basic Alumina (11 g) Column was used to separate the target compounds into two separate fractions. The sample extract was loaded onto the PPS system where it flows through the first ABN column and then onto the carbon/celite column. The PBDE / dioxin like (coplanar) PCB fraction was further eluted onto the basic alumina column for additional clean-up and is collected. The carbon column was then back-flushed with toluene to collect the PCNs, PCDD/Fs, and coplanar PCBs. Prior to analysis 20 µL of injection standard comprising of $^{13}C_{10}$-CN-64 (Cambridge Isotope Laboratories, Andover, MA) was added to the PCN containing fraction.

Analysis

The samples were analysed for PCNs using GC-HRMS on a Micromass Autospec Ultima Premier HRMS (Waters Corporation, Milford, MA) coupled to a Hewlett-Packard (Agilent Technologies, Wilmington, DE) HP7890 N GC fitted with a 40 m RTX-Dioxin2 column (0.18 mm i.d., 0.18 µm film thickness, Restek). The GC-HRMS system was tuned to >10,000 resolving power (10% valley definition) and samples were injected in the splitless mode with a He carrier gas at a flow rate of 0.8 mL/min, and the injector and transfer line temperatures were maintained at 250 and 280 °C, respectively. The GC program was set at an initial temperature of 110°C (hold 1 min), then ramped at a rate of 25 C min^{-1} to 200 °C (hold 5 min), then at 2.5 °C min^{-1} to 235 °C (hold 3 min), then 3 °C min^{-1} to 267 °C, and finally at 10 °C min^{-1} to 300 °C. The GC-HRMS was operated using selected ion monitoring (SIM) to monitor the ions listed in Table 1. Perfluorokerosene (PFK) was used as a reference compound for lock mass to adjust for magnet drift. PCN congeners were quantified based on $^{13}C_{10}$-labelled surrogates (isotope dilution) or internal standard responses.

Quality Control

The requirements for positive identification of PCN congeners included: elution of the specific congener in the required time window, the chromatographic peak must have a Gaussian shape, isotopic peaks must maximize within ±2 seconds of each other and ±2 seconds of the $^{13}C_{10}$-labelled surrogate if available, isotope ratio of the two peaks in the sample must be within ±15% of the theoretical isotope ratios listed in Table 1, and the calculated concentration must be greater than 5 times the blank value (blank values were comparable to the MDLs reported in Tables 2&3).

Blanks consisted of a blank fish matrix (Alaskan Pollock, *Theragra chalcogramma*) or a soil/sediment matrix (Ottawa sand). Blank values were generated by integrating analyte peaks or based on an estimated signal to noise ratio of 3:1. In total 34 performance and recovery (PAR) spiked blank fish matrix (Alaskan Pollock) samples and 28 PAR spiked blank sediment (Ottawa sand) samples were analysed. The linearity of the method for all congeners was confirmed over the range of the calibration standards (1.65 pg to 620 pg on column).

Table 1. Method selected ion monitoring (SIM) settings and theoretical ion ratios

Function Group	Quantitation Ions (m/z)	Compound	Dwell (ms)	Delay (ms)	Theoretical Isotopic Ratio	Acceptable Range (±15%)
	162.0236*, 164.0207	Mono-CNs	200	10	3.03	2.58-3.48

1	195.9847*, 197.9817	Di-CNs	200	10	1.53	1.30-1.76
	192.9888	PFK Lock Mass	30	10		
2	229.9457*, 231.9427	Tri-CNs	175	10	1.02	0.87-1.17
	265.9038*, 263.9067	Tetra-CNs	175	10	1.30	1.11-1.5
	275.9373*, 273.9403	$^{13}C_{10}$ Tetra-CNs	50	10	1.30	1.11-1.5
	268.9824	PFK Lock Mass	30	10		
3	299.8648*, 297.8677	Penta-CNs	200	10	1.62	1.38-1.86
	309.8983*, 307.9013	$^{13}C_{10}$ Penta-CNs	50	10	1.62	1.38-1.86
	292.9824**	PFK Lock Mass	30	10		
4	333.8258*, 335.8229	Hexa-CNs,	200	10	1.23	1.05-1.41
	343.8594*, 345.8564	$^{13}C_{10}$ Hexa-CNs	50	10	1.23	1.05-1.41
	342.9792	PFK Lock Mass	30	10		
5	367.7868*, 369.7839	Hepta-CNs,	200	10	1.02	0.87-1.17
	377.8204*, 379.8174	$^{13}C_{10}$ Hepta-CNs	50	10	1.15	0.87-1.17
	380.9760	PFK Lock Mass	30	10		
6	403.7449*, 401.7479	Octa-CNs,	200	10	1.14	0.97-1.31
	413.7785*, 411.7814	$^{13}C_{10}$ OctaCNs	50	10	1.15	0.97-1.31
	392.9760	PFK Lock Mass	30	10		

* Ion occurs at greatest abundance in molecular ion cluster

** Quantification ion (m/z) 304.9824 may be used as an alternative PFK lock mass

Results and discussion

PCNs in spiked fish and sediment

The results from the spiked fish samples are presented in Table 2 and the results from the spiked sediment samples in Table 3. The method was validated for 24 PCNs, with eight of the PCNs (CN-52&60, CN-66&67, CN-64&68, CN-71&72) occurring as co-eluting isomers which are reported as a combination of the two. As new analytical standards become available they can be added to the method to potentially improve accuracy and provide individual concentration data for more congeners. The separation achieved using the RTX-Dioxin2 column and settings specified in the experimental section is displayed in Figure 1, which shows selected ion chromatograms obtained from a natural fish sample.

Instrument detection limits (IDLs) were determined from replicate lowest level standard injections, and is the relative standard deviation (RSD) multiplied by the T-value for 8 injections. The method detection limit was calculated by multiplying the T-value by the RSD for the respective fortified matrix blanks of the within run data sets. The IDL for each PCN was between 0.06 and 0.13 pg (on column), the MDL for the fish extracts was 1.3 to 3.4 pg/g (wet weight) (Table 2) and 0.46 to 1.2 pg/g (dry weight) for the sediment (Table 3). These detection limits are effective for the determination of PCNs in tissue, soils and sediments as concentrations reported all over the globe such as in; Sweden (Jarnberg et al. 1997), China (Zhang et al. 2015), Pakistan (Mahmood et al. 2014) and the Great Lakes region (Clement et al. 2012; Helm et al. 2006) generally range from low pg/g to ng/g.

The accuracy and precision recorded throughout the experimental period are presented in Table 2 and Table 3. For the spiked tissue samples the average accuracy (% Target value) for the 24 measured PCNs was 98% for the samples analysed within one run and 100% for the between run samples, with an average precision (%RSD) of 4.5% and 12% respectively. Similarly, for the spiked sediment samples, the average accuracy (% Target) for the 24 measured PCNs was 104% for the samples analysed within one run and 104% for the between run samples, with an average precision (%RSD) of 4.4% and 12%, respectively. The results are consistent for both the within and between run samples in different matrices indicating that the method is robust and suitable for routine analysis.

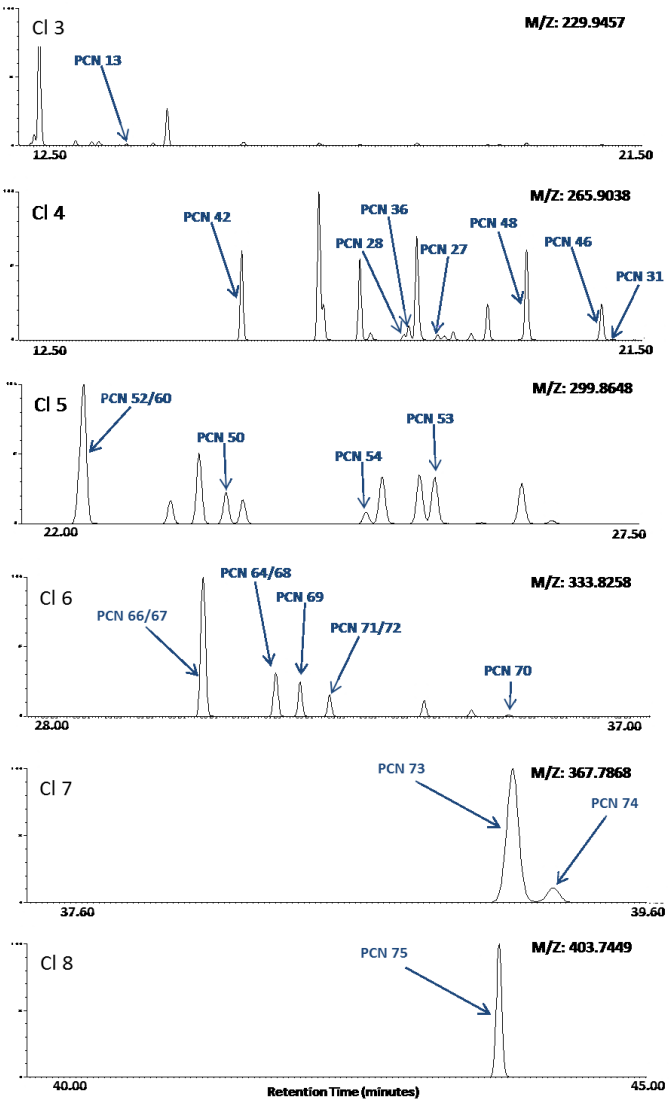


Figure 1 SIM chromatograms displaying separation of PCNs in a natural fish sample. Labelled peaks represent the individual congeners for which certified standards are available, and for the target congener list in this accredited PCN method

Table 2. Method performance data for fortified blank fish samples

Compound (Cl substitution)	Within one Run (n=9)				Between Runs (n=34)				Instrument Detection Limits (pg)	Method Detection Limits (pg/g)
	Spiked amount (pg/g)	Mean (pg/g)	% Target	% RSD	Spiked amount (pg/g)	Mean (pg/g)	% Target	% RSD		
CN-13 (1,2,3)	16	17	110	4.2	94	91.	97	11	0.13	2.1
CN-27 (1,2,3,4)	16	16	102	4.1	96	95	99	10	0.09	1.9
CN-28 (1,2,3,5)	15	16	104	7.3	94	96	102	11	0.12	3.3
CN-31 (1,2,3,8)	16	14	89	4.3	96	96	100	18	0.08	2.5
CN-36 (1,2,5,6)	16	16	103	5.4	91	90	99	12	0.09	1.7
CN-42 (1,3,5,7)	16	18	111	2.5	97	96.	99	10	0.09	1.3
CN-46 (1,4,5,8)	16	15	93	5.9	96	97	101	20	0.07	2.5
CN-48 (2,3,6,7)	16	15	97	3.3	94	101	105	12	0.10	1.5
CN-50 (1,2,3,4,6)	16	15	96	2.9	96	95	99	11	0.07	1.3
CN-52/60 (1,2,3,5,7 / 1,2,4,6,7)	16	14	92	4.7	94	96	102	11	0.10	1.9
CN-53 (1,2,3,5,8)	16	16	98	4.7	96	95	99	12	0.09	2.1
CN-54 (1,2,3,6,7)	16	16	102	3.7	96	97	101	11	0.06	1.7
CN-64/68 (1,2,3,4,5,7 / 1,2,3,5,6,8)	16	15	95	4.0	96	100	96	12	0.07	1.7
CN-66/67 (1,2,3,4,6,7 / 1,2,3,5,6,7)	16	14	89	3.2	94	95	101	10	0.09	1.3
CN-69 (1,2,3,5,7,8)	16	15	97	4.6	96	96	100	11	0.08	2.1
CN-70 (1,2,3,6,7,8)	16	16	104	3.2	96	101	105	12	0.08	1.5
CN-71/72 (1,2,4,5,6,8 / 1,2,4,5,7,8)	16	15	93	4.5	96	93	97	13	0.07	1.9
CN-73 (1,2,3,4,5,6,7)	16	15	97	4.0	94	96	102	12	0.06	1.7
CN-74 (1,2,3,4,5,6,8)	16	14	88	8.4	96	87	90	15	0.08	3.4
CN-75 (1,2,3,4,5,6,7,8)	16	15	97	5.2	94	96	102	11	0.06	2.3
Average			98	4.5			100	12	0.084	2.0

Table 3. Method performance data for fortified sediment samples

Compound (Cl substitution)	Within one Run (n=8)				Between Runs (n=28)				Instrument Detection Limits (pg)	Method Detection Limits (pg/g)
	Spiked amount (pg/g)	Mean (pg/g)	% Target	% RSD	Spiked amount (pg/g)	Mean (pg/g)	% Target	% RSD		
CN-13 (1,2,3)	9.13	9.6	105	5.5	94	103	110	32	0.13	0.56
CN-27 (1,2,3,4)	9.32	9.1	98	2.2	96	99	103	10	0.09	0.55
CN-28 (1,2,3,5)	8.84	8.8	100	5.6	94	99	105	10	0.12	1.00
CN-31 (1,2,3,8)	9.32	10	107	5.3	96	96	100	11	0.08	0.94
CN-36 (1,2,5,6)	9.13	8.7	95	5.4	91	99	108	12	0.09	1.2
CN-42 (1,3,5,7)	9.42	9.3	99	3.5	97	98	101	14	0.09	0.46
CN-46 (1,4,5,8)	9.32	10	110	5.2	96	99	103	14	0.07	0.82
CN-48 (2,3,6,7)	9.32	10	108	4.4	94	99	105	12	0.10	0.87
CN-50 (1,2,3,4,6)	9.32	9.9	107	4.2	96	99	103	7.0	0.07	0.63
CN-52/60 (1,2,3,5,7 / 1,2,4,6,7)	9.13	9.2	101	4.2	94	99	105	6.7	0.10	0.77
CN-53 (1,2,3,5,8)	9.32	11	115	4.2	96	95	99	18	0.09	1.0
CN-54 (1,2,3,6,7)	9.32	11	120	3.5	96	95	99	16	0.06	1.1
CN-64/68 (1,2,3,4,5,7 / 1,2,3,5,6,8)	9.32	9.4	101	4.9	96	99	103	8.1	0.07	0.81
CN-66/67 (1,2,3,4,6,7 / 1,2,3,5,6,7)	9.13	9.0	99	3.4	94	100	106	7.0	0.09	0.73
CN-69 (1,2,3,5,7,8)	9.32	9.5	102	3.2	96	99	103	7.9	0.08	0.63
CN-70 (1,2,3,6,7,8)	9.32	11	114	4.9	96	98	102	20	0.08	1.1
CN-71/72 (1,2,4,5,6,8 / 1,2,4,5,7,8)	9.32	9.4	101	4.0	96	98	102	9.7	0.07	0.71
CN-73 (1,2,3,4,5,6,7)	9.13	9.5	104	5.1	94	101	107	9.0	0.06	0.87
CN-74 (1,2,3,4,5,6,8)	9.32	8.5	91	5.3	96	101	105	9.0	0.08	0.65
CN-75 (1,2,3,4,5,6,7,8)	9.13	9.6	105	5.3	94	100	106	4.5	0.06	0.70
Average			104	4.4			104	12	0.084	0.81

Method Applications and Performance

Spiked samples were used in this assessment due to the lack of a certified reference material for PCNs. An interlaboratory study was undertaken by Harner and Kucklick (2003), however the authors used dilutions of a Halowax solution and did not include measurements of PCNs in an environmental matrix. NIST SRM 1941a (now 1941b) Organics in Marine Sediment was proposed as a suitable reference material by Harner and Kucklick (2003), however validated congener specific data has not been

released. The validation of such a certified reference material for individual PCN congeners would be highly beneficial. Despite the lack of a certified reference material, the Northern Contaminants Program (NCP) inter-laboratory studies involved the determination PCNs (Myers et al. 2015). Samples included an injection-ready standard for which concentrations were known and several natural matrix samples with unknown concentrations. The method presented here was applied to the NCP injection-ready standard and the results (Figure 2) show good concordance with the reference concentrations for all congeners reported.

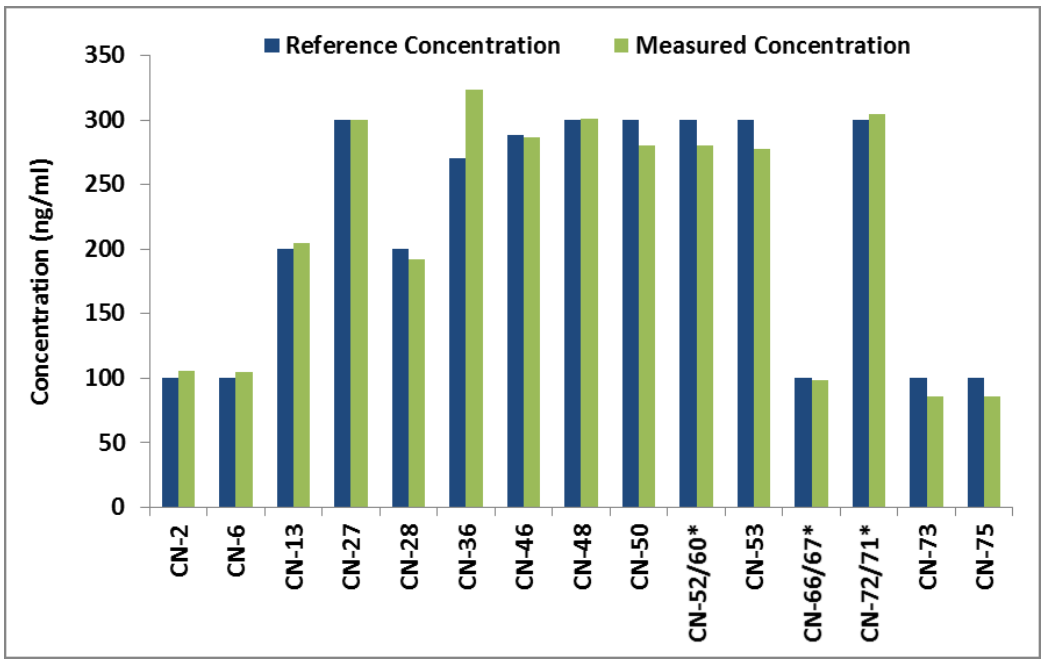


Figure 2 Performance of method against injection-ready standard from Northern Contaminants Program inter-laboratory study (* CN-60, 67 and 71 are co eluting congeners not present in the analysed standard)

Lake Ontario, one of the Laurentian Great Lakes, is subject to multiple inputs from a large population and commercial/industrial base along its western and north-western shores. The method was used to determine PCN concentrations in 10 sediment samples from a background site in eastern Lake Ontario (Figure 3). Analyte recoveries from these samples were good and generally ranged from 60 to 100% for the ^{13}C labelled surrogates with an average of 70%. To provide a comparison, 10 sediment samples were also obtained from an industrialised area in the Lake Ontario region that is known to have elevated levels of PCNs relative to background (Figure 4). Analyte recoveries in these higher level samples generally ranged from 80 to 120% for the ^{13}C labelled surrogates with an average of 94%. Concentrations of PCNs in sediments from the industrialised region were approximately 10 to 100 times greater than concentrations in the background location. The major congeners identified in the background region were CN-52/60, CN-66/67 and CN-74, whereas in the industrialised region CN-42 and CN-46 were present in the highest concentrations.

In addition to the sediment samples, PCN concentrations were determined for tissue samples (fillets with skin removed) of five different fish species obtained from Lake Ontario (Figure 5). This included Bluegill (*Lepomis macrochirus*), Brown bullhead (*Ameiurus nebulosus*), Channel catfish (*Ictalurus punctatus*), Northern pike (*Esox lucius*) and Pumpkinseed (*Lepomis gibbosus*). Sample recoveries generally ranged from 70 to 110% for the ^{13}C labelled surrogates with an average of 87%. Congeners present in the highest concentrations in the fish included CN-42, CN-52/60 which have previously

shown a high bioaccumulation potential (Helm et al., 2008; Gewurtz et al., 2009). The concentrations of CN-46 were surprisingly low given the high values in the sediment, possibly indicating that this congener may be more susceptible to biotransformation and elimination (Gewurtz et al., 2009). The highest PCN concentrations were found in the catfish and bullhead which are benthic fish (living and feeding in the sediment), and the pike which is at a higher tropic level of the food chain. These factors are likely to have contributed to the higher values reported in these species.

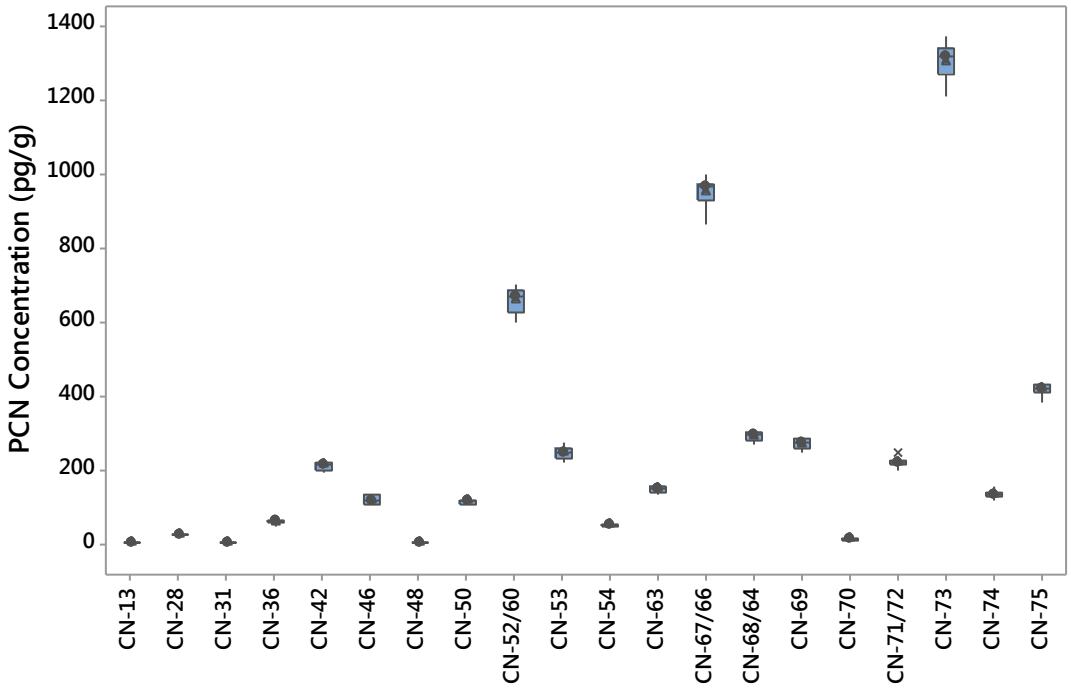
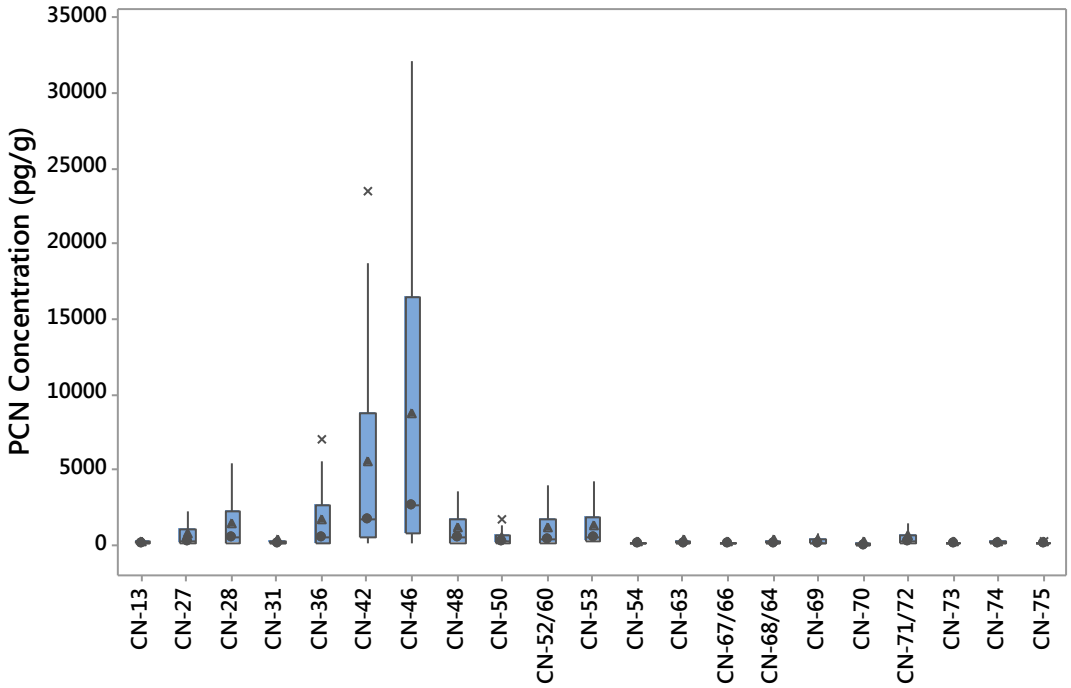
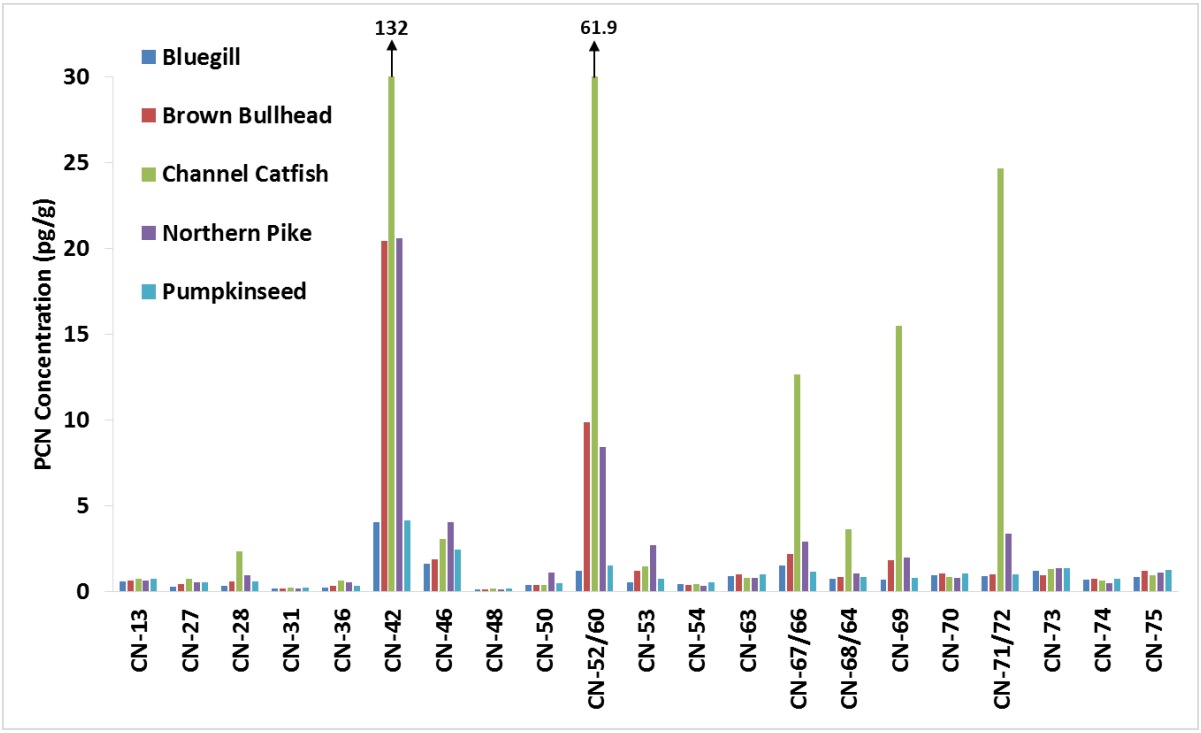


Figure 3. Concentrations of PCNs present above LODs from 10 sediment samples obtained from a background region of Lake Ontario. The interquartile range is displayed by the box, the mean by the triangle, the median by the circle and any outliers are identified by the "x",



304

305 **Figure 4. Concentrations of PCNs present above LODs from 10 sediment samples obtained from an**
306 **industrialised region of Lake Ontario. The interquartile range is displayed by the box, the mean by**
307 **the triangle, the median by the circle and any outliers are identified by the "x",**



308

309 **Figure 5. Concentrations of PCNs present above LODs in tissue (fillets with skin removed) from five**
310 **different fish species caught from Lake Ontario**

In addition to quantifying the individual congeners listed in Table 2 & 3, the method can be used to determine total PCN concentrations for each homologue by summing the remaining non-target PCN congeners in each spectra. The 24 individual congeners targeted in this method included the PCNs having dioxin-like toxicity as well as those with the greatest bioaccumulation potential (e.g. PCN-42, 52, 60, 66, 67, 73) (Helm et al., 2008; Gewurtz et al., 2009).

Previous data from Helm et al. 2008 and Gewurtz et al. 2009 have identified that, the sum of the 24 target PCNs accounted for 83% ± 10% of total PCNs (range 57-92%) in Lake trout from Lake Ontario. They also identified that in sediment, the target congeners tend to account for a slightly lower percentage of total PCNs. Using data from the Great Lakes as examples, the contribution of the target PCN congeners to total PCN in surface sediments from Lakes Erie and Ontario averaged 46% ± 10% 1σ (range 34-73%) and 57% ± 12% 1σ (range 37-81%), respectively. Data from this current study was similar with these previous studies as the contribution of the target PCNs to the total in the background region was 64% 1.8%,1σ (range 62-68%), however in the industrialised region the contribution was 15% ± 12% 1σ (range 9.8-28%). The contribution of the target PCNs to the total in the fish was 52, 47 and 50% for the bullhead, catfish and pike respectively (concentrations in the bluegill and pumpkinseed were predominantly below LOD so no assessment was made). This highlights the importance of this method which provides not only detailed congener-specific analysis using certified standards for TEQ calculations, but also the quantification of the remaining congeners (based on exact mass) to calculate total PCN concentrations.

Conclusions

The method presented here allows for the determination of PCNs in soils, sediments and tissue. The method was tested on 34 spiked fish tissue and 28 spiked sediment samples that were analysed by GC-HRMS. The method was capable of producing IDLs for each PCN at between 0.06 and 0.13 pg (on column), whereas the MDL for the fish extracts ranged from 1.3 to 3.4 pg/g (wet weight) and 0.46 to 1.2 pg/g for sediment. The method produced excellent accuracy and precision. The average accuracy of 34 spiked fish samples analysed over a period of several months was 100% with a precision (%RSD) of 12%. This was also similar for 28 spiked sediment samples where the average accuracy was 104% and precision (%RSD) was 12%. There is currently no certified reference material for PCNs however the method was tested on an injection-ready standard obtained from Northern Contaminants Program inter-laboratory study. The congener specific results for all 15 measured PCNs were within 20% of the reported values, with 12 of the 15 within 10%. The application of the method to environmental samples was demonstrated through the analysis of fish and sediment samples from a background and industrialised region of Lake Ontario, Canada.

The method presented is one of the most comprehensive and accurate congener-specific methods available and is capable of providing quantitative data for the most toxic PCNs (with TEFs) and the most prevalent PCNs at environmentally relevant concentrations. It was developed based on an existing method for dioxins and furans (MOECC 3418) with only minor modifications, and so can be easily adopted by laboratories already analysing dioxins and furans. This has practical applications such as increasing laboratory throughput and reducing costs as samples produced through one extraction method have the potential to be used for the determination of additional compounds.

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